

## PROMOTER-DEBUGGING MODULE: LAB NOTEBOOK WEEKLY SUMMARY

### Week 1 (1/6/2015-5/6/2015)

1. Summer training, mainly for recalling the basic techniques
2. Discussion for the plan of the main project

### Week 2 (8/6/2015-12/6/2015)

1. Transformation of pSB1C3-BBa\_I0500, pSB1C3-BBa\_B0015, pSB1C3-BBa\_B0030, pSB1C3-BBa\_K381001 and pSB1C3-BBa\_E0840
2. Inoculation of pSB1C3-BBa\_I0500, pSB1C3-BBa\_B0015, pSB1C3-BBa\_B0030, pSB1C3-BBa\_K381001 and pSB1C3-BBa\_E0840
3. Plasmid extraction of pSB1C3-BBa\_I0500, pSB1C3-BBa\_B0015, pSB1C3-BBa\_B0030, pSB1C3-BBa\_K381001 and pSB1C3-BBa\_E0840
4. PCR cloning of *phoR* from *E. coli* strain DH10B
5. Digestion of pSB1C3-BBa\_J04450
6. Ligation of *phoR* with pSB1C3 and pSB1C3-BBa\_I0500 with pSB1C3-BBa\_B0030
7. Colony PCR for identifying candidate colonies for pSB1C3-*phoR*

### Week 3 (15/6/2015-19/6/2015)

1. Transformation of pSB1A2-BBa\_B0030, pSB1A2-BBa\_E0840, pSB1AK3-BBa\_B0015
2. PCR reaction for identifying candidate colonies for pSB1C3-*phoR* and pSB1C3-BBa\_I0500-B0030
3. Digestion of pSB1C3-BBa\_J04450
4. Sequencing of pSB1C3-*phoR*

### Week 4 (22/6/2015-26/6/2015)

1. Colony PCR for checking the identity of pSB1C3-*phoR* candidate 7 and 8 by VF<sub>2</sub> and VR primers
2. Restriction check of pSB1C3-*phoR* candidate 7 and 8 using BstEII
3. PCR cloning of *nsrR* from *E. coli* strain DH10B

4. Ligation of *nsrR* with pSB1C3
5. Transformation of pSB1C3-*nsrR*

### **Week 5 (29/6/2015-3/7/2015)**

1. New plan for the constructs: instead of having constructs of pSB1C3-*nsrR* and pSB1C3-*phoR* for sequencing, rather, ligate *nsrR* and *phoR* with pSB1C3-BBa\_B0030, respectively.
2. Digestion of pSB1C3-BBa\_B0030.
3. PCR cloning of *nsrR* and *phoR* from *E. coli* strain DH10B.
4. Digestion of PCR product of *nsrR* and *phoR*.
5. Ligation of pSB1C3-BBa\_B0030 with *nsrR* and *phoR* respectively.
6. Digestion of pSB1C3-BBa\_J04450.
7. Transformation of pSB1C3-BBa\_B0030-*nsrR* and pSB1C3-BBa\_B0030-*phoR*.
8. Colony PCR for checking the identity of pSB1C3-BBa\_B0030-*nsrR*, pSB1C3-BBa\_B0030-*phoR* candidate colonies. Identified candidate 1 and 5 of pSB1C3-BBa\_B0030-*phoR*.
9. Confirmed the identity of candidate 1 and 5 of pSB1C3-BBa\_B0030-*phoR* by restriction check using PvuII, ready for sequencing.
10. Digestion of pSB1C3-BBa\_B0030
11. Ligation of pSB1C3-BBa\_B0030 with *nsrR*.
12. Transformation of pSB1C3-BBa\_B0030-*nsrR*.
13. Digestion of pSB1A2-BBa\_E0840

### **Week 6 (6/7/2015-10/7/2015)**

1. Send pSB1C3-BBa\_B0030-*phoR* candidate 5 for sequencing.
2. Ligation of pSB1C3-BBa\_B0030 with *nsrR*.
3. Transformation of pSB1C3-BBa\_B0030-*nsrR*.
4. Colony PCR of pSB1C3-BBa\_B0030-*nsrR* candidate 1-22.
5. Identified candidate 3 and 13 of pSB1C3-BBa\_B0030-*nsrR* by colony PCR.
6. Restriction check of pSB1C3-BBa\_B0030-*nsrR* candidate 3 and 13 by *Scal*.
7. Identified pSB1C3-BBa\_B0030-*nsrR* candidate 13 by restriction check.

8. Send pSB1C3-BBa\_B0030-*nsrR* candidate 13 for sequencing.
9. Miniprep of pSB1C3-BBa\_B0030-*phoR* candidate 5, 7, 8, pSB1C3-BBa\_B0030-*nsrR* candidate 3.
10. Sequencing result of pSB1C3-BBa\_B0030-*phoR* candidate 5 showed missing of BBa\_B0030.
11. Inoculation and streak pSB1C3-BBa\_B0030, pSB1AK3-BBa\_B0015

### **Week 7 (13/7/2015-17/7/2015)**

1. Send pSB1C3-BBa\_B0030, pSB1C3-BBa\_B0030-*phoR* candidate 1 for sequencing.
2. From sequencing result of pSB1C3-BBa\_B0030, it showed extra nucleotides before and after the BBa\_B0030 sequence, which is inconsistent with the sequence from the iGEM Parts Registry.
3. Transformation of pSB1C3-BBa\_B0032.
4. Miniprep of pSB1C3-BBa\_B0032 and pSB1C3-BBa\_B0030.
5. Digestion of pSB1C3-BBa\_B0032.
6. Ligation of pSB1C3-BBa\_B0032 with *phoR* and *nsrR* separately.
7. Transformation of pSB1C3-BBa\_B0032-*nsrR* and pSB1C3-BBa\_B0032-*phoR*.
8. Colony PCR of pSB1C3-BBa\_B0032-*nsrR* candidate 1-8 and pSB1C3-BBa\_B0032-*phoR* candidate 1-8.
9. Restriction check of pSB1C3-BBa\_B0032-*nsrR* candidate 5 and pSB1C3-BBa\_B0032-*phoR* candidate 6.
10. Glycerol stock for pSB1AK3-BBa\_B0015.
11. Functional assay for *yeaRp*.
12. Characterization of *yeaRp*.
13. Prepared modified M9 minimal medium for *yeaRp* characterization with NH<sub>4</sub>Cl being replaced by MOPS salt for testing the viable range.

### **Week 8 (20/7/2015-24/7/2015)**

1. Digestion of *nsrR*, *phoR* PCR product and pSB1C3-BBa\_I0500-B0030.
2. Ligation of *nsrR* and *phoR* with pSB1C3-BBa\_I0500-B0030 separately.
3. Transformation of pSB1C3-BBa\_I0500-B0030-*nsrR* and pSB1C3-BBa\_I0500-B0030-*phoR*.

4. Colony PCR for identifying candidates of pSB1C3-BBa\_I0500-B0030-*nsrR* and pSB1C3-BBa\_I0500-B0030-*phoR*.
5. Functional assay of pSB1C3-BBa\_K381001 with 0mM and 20mM KNO<sub>3</sub>.
6. Characterization of pSB1C3-BBa\_K381001 with series of 0mM, 5mM, 10mM, 15mM, 20mM, 50mM, 100mM KNO<sub>3</sub>.
7. Characterization of pSB1C3-BBa\_K381001 with series of 0mM, 2mM, 4mM, 6mM, 8mM, 10mM KNO<sub>3</sub>.
8. Ligation of *nsrR* and *phoR* with pSB1C3-BBa\_I0500-B0030 separately. (new)
9. Transformation of pSB1C3-BBa\_I0500-B0030-*nsrR* and pSB1C3-BBa\_I0500-B0030-*phoR*. (new)

### **Week 9 (27/7/2015-31/7/2015)**

1. Characterization of pSB1C3-BBa\_K381001 in M9 minimal medium with 0μM, 20μM, 200μM, 2000μM KNO<sub>3</sub>.
2. Characterization of pSB1C3-BBa\_K381001 in LB for 3 trials with 0mM, 2mM, 4mM, 6mM, 8mM, 10mM KNO<sub>3</sub>. (Followed directly the protocol from BCCS-Bristol 2010)

As the constructs of pSB1C3-BBa\_I0500-B0030-*nsrR* and pSB1C3-BBa\_I0500-B0030-*phoR* were stuck for 2 months in the cloning process, some trouble-shoots were adopted as follows: (point 1-9)

3. PCR cloning of *nsrR* and *phoR* from DH10B, with 4 tubes of 50μl reaction.
4. PCR purification using small column, 5μl after 5μL for eluting the sample.
5. Digestion of *nsrR* and *phoR* with XbaI and PstI-HF digestion enzymes in 37°C incubator for 2 hrs.
6. PCR purification using small column, 5μl after 5μL for eluting the sample.
7. Ligation of *nsrR* with pSB1C3-BBa\_I0500-B0030 and *phoR* with pSB1C3-BBa\_I0500-B0030 for 3 hrs in 16°C incubator.
8. Transformation of pSB1C3-BBa\_I0500-B0030-*nsrR* and pSB1C3-BBa\_I0500-B0030-*phoR*.
9. Colony PCR of pSB1C3-BBa\_I0500-B0030-*nsrR* candidate 1-26 and pSB1C3-BBa\_I0500-B0030-*phoR* candidate 1-26.

### **Week 10 (3/8/2015-7/8/2015)**

1. Restriction check on pSB1C3-BBa\_I0500-B0030-*nsrR* candidate 19-23, and 26.

2. Sent pSB1C3-BBa\_I0500-B0030-nsrR candidate 23 for sequencing
3. Characterization of pSB1C3-BBa\_K381001 using LB medium for trial 4-6 with concentration gradient of 0, 2, 4, 6, 8, 10, 15, 20mM KNO<sub>3</sub>
4. Characterization of pSB1C3-BBa\_K381001 using M9 minimal medium for trial 1-3 with concentration gradient of 0, 2, 4, 6, 8, 10mM KNO<sub>3</sub>
5. PCR cloning of *phoR* from DH10B
6. Digestion of *phoR*.
7. Ligation of *phoR* with pSB1C3-BBa\_I0500-B0030 (*P<sub>bad</sub>*-RBS)
8. Transformation of pSB1C3-BBa\_I0500-B0030-*phoR*.
9. Colony PCR of pSB1C3-BBa\_I0500-B0030-*phoR*.

### **Week 11 (10/8/2015- 14/8/2015)**

1. Sent pSB1C3-BBa\_I0500-B0030-nsrR candidate 23 for sequencing
2. Characterization of pSB1C3-BBa\_K381001 using LB medium for trial 8 with concentration gradient of 0, 2, 4, 6, 8, 10, 15, 20mM KNO<sub>3</sub>
3. Characterization of pSB1C3-BBa\_K381001 using M9 minimal medium for trial 4-6 with concentration gradient of 0, 2, 4, 6, 8, 10mM KNO<sub>3</sub>
4. Characterization of pSB1C3-BBa\_K381001 using M9 minimal medium for trial 1-5 with concentration gradient of 0, 20, 200, 500, 1000, 2000μM KNO<sub>3</sub>
5. Restriction check of pSB1C3-BBa\_I0500-B0030-*phoR* candidate 6-10 using enzyme BstEI
6. Sent pSB1C3-BBa\_I0500-B0030-*phoR* candidate 9 for sequencing

### **Week 12 (17/8/2015- 23/8/2015)**

1. Ligate pSB1C3-BBa\_I0500-B0030-*nsrR* with pSB1C3-BBa\_K381001
2. Identifying correct candidates for pSB1C3-BBa\_I0500-B0030-*nsrR*-K381001 by restriction check and colony PCR.
3. Characterization of *yeaRp* promoter in M9 minimal medium with concentration of 0-500μM, and in LB with concentration of 0-50mM.
4. Gibson Assembly on pSB1C3-BBa\_I0500-B0030-*phoR*-B0015 construct.
5. Identifying candidates for pSB1C3-BBa\_I0500-B0030-*phoR*-B0015 construct using colony PCR.

### **Week 13 (24/8/2015-30/8/2015)**

1. Ligate pSB1C3-BBa\_I0500-B0030-*nsrR* with pSB1C3-BBa\_K381001
2. Identifying correct candidates for pSB1C3-BBa\_I0500-B0030-*nsrR*-K381001 by restriction check and colony PCR.
3. Confirmed pSB1C3-BBa\_I0500-B0030-*nsrR*-K381001 candidate 6 is correct.
3. Characterization of *yeaRp* promoter in M9 minimal medium with concentration of 0-500 $\mu$ M.
4. Gibson Assembly on pSB1C3-BBa\_I0500-B0030-*phoR*-B0015 construct.
5. Identifying candidates for pSB1C3-BBa\_I0500-B0030-*phoR*-B0015 construct using colony PCR and restriction check.
6. Functionality assay on the pSB1C3-BBa\_I0500-B0030-*nsrR*-K381001, the debug method with *yeaRp* promoter.

### **Week 14 (31/8/2015-6/9/2015)**

1. Characterization on the pSB1C3-BBa\_I0500-B0030-*nsrR*-K381001, the debug method with *yeaRp* promoter with M9 minimal medium from 0- 2mM nitrate and 10mM arabinose concentration.